Client: Example Client ABC123
123 Test Drive

UNITED STATES

Physician: Doctor, Example

Patient: Patient, Example
DOB
Sex:
Patient Identifiers: 01234567890ABCD, 012345
Visit Number (FIN): 01234567890ABCD
Collection Date: 01/01/2017 12:34

Immunobullous Disease Antibody Panel

ARUP test code 3001409

EER Immunobullous Disease Panel

See Note

Authorized individuals can access the ARUP Enhanced Report using the following link:

https:

Immunobullous Disease Panel

See Note

CLINICAL INFORMATION
Widespread blisters and erosions, urticarial lesions, mucosal involvement. Presumptive diagnosis is immunobullous disease.

Specimen Details
S22-IP0000506 - Serum; Collected: ; Received:

DIAGNOSTIC INTERPRETATION

Consistent with pemphigoid; positive IgG, including IgG4, basement membrane zone antibodies, epidermal (roof) localization with split skin substrate, and negative IgA basement membrane zone antibodies by indirect immunofluorescence with increased IgG BP180 and IgG BP230 antibody levels and normal IgG type VII collagen antibody level by ELISAs, and negative/normal IgG, including IgG4, and IgA cell surface (pemphigus) antibodies

(See Results and Comments)

RESULTS
Indirect Immunofluorescence (IIF)

Basement Membrane Zone (BMZ) IgG, IgG4, and IgA Antibodies

IgG: Positive, titer greater than 1:40,960 (H), monkey esophagus substrate
Positive, epidermal pattern (roof), titer 1:40,960 (H), human split skin substrate

IgG4: Positive, titer greater than 1:40 (H), monkey esophagus substrate
Positive, epidermal pattern (roof), titer greater than 1:40 (H), human split skin substrate

IgA: Negative, monkey esophagus substrate
Negative, human split skin substrate

H=High, L=Low, *=Abnormal, C=Critical

```
        Reference Range:
          Negative - Titer less than 1:10
          Borderline - Titer 1:10
          Positive (H) - Titer greater than 1:10

        Localization Pattern on Human BMZ Split Skin:
          Epidermal (roof) or combined epidermal-dermal
          (roof and floor) IgG and/or IgG4 BMZ antibodies
          = pemphigoid (including pemphigoid gestationis,
          bullous pemphigoid, mucous membrane pemphigoid)

          Dermal (floor) IgG and/or IgG4 BMZ antibodies =
          epidermolysis bullosa acquisita or bullous lupus
          erythematosus or anti-laminin-332 pemphigoid or
          anti-p200 (laminin gamma-1) pemphigoid or another
          rare pemphigoid subtype

          Epidermal (roof), combined epidermal-dermal (roof
          and floor), or, dermal (floor) IgA BMZ antibodies =
          linear IgA disease (including linear IgA bullous
          dermatosis and chronic bullous disease of childhood)

       (H) = high/positive

---------------------------------
Cell Surface (CS)/Intercellular Substance (ICS) IgG, IgG4,
and IgA Antibodies

IgG:  Negative, monkey esophagus substrate
      Negative, intact human skin substrate

    IgG4:  Negative, monkey esophagus substrate
           Negative, intact human skin substrate

IgA:  Negative, monkey esophagus substrate
      Negative, intact human skin substrate

      Reference Range:
        Negative - Titer less than 1:10
        Borderline - Titer 1:10
        Positive (H) - Titer greater than 1:10

     (H) = high/positive

Enzyme-Linked Immunosorbent Assay (ELISA)
-----------------------------------------
Bullous Pemphigoid (BP)180 and BP230 IgG Antibodies

IgG BP180 antibody level:  14 U/mL (H)

    Reference Range:
           Normal (negative) = Less than 9 U/mL
    Increased (H) (positive) = 9 U/mL and greater

IgG BP230 antibody level:  83 U/mL (H)

    Reference Range:
           Normal (negative) = Less than 9 U/mL
    Increased (H) (positive) = 9 U/mL and greater

-----------------------------------------
Type VII Collagen IgG Antibodies

IgG type VII collagen antibody level:  2 U/mL

    Reference Range:
                  Normal (negative) = Less than 7 U/mL
Slightly increased (H) (positive) = 7-8 U/mL
         Increased (H) (positive) = 9 U/mL and greater
```

Desmoglein (DSG) 1 and 3 IgG Antibodies

IgG desmoglein 1 antibody level: 0 U/mL

Reference Range:
Normal (negative) = Less than 14 U/mL
Borderline/Indeterminate = 14-20 U/mL
Increased (H) (positive) = Greater than 20 U/mL

IgG desmoglein 3 antibody level: 2 U/mL

Reference Range:
Normal (negative) = Less than 9 U/mL
Borderline/Indeterminate = 9-20 U/mL
Increased (H) (positive) = Greater than 20 U/mL

COMMENTS

Specific

The findings with this testing, demonstrating positive IgG, including IgG4 basement membrane zone antibody reactivity with monkey esophagus substrate and epidermal localization (roof) on human split skin substrate (also known as salt split skin) by indirect immunofluorescence and increased IgG BP180 and IgG BP230 antibody levels by ELISAs, support the diagnosis of pemphigoid. The IgG type VII collagen antibody level is normal by ELISA, and, together with the lack of dermal localization of IgG basement membrane zone antibody reactivity on split skin substrate by indirect immunofluorescence, is against the diagnosis of epidermolysis bullosa acquisita. Although IgA basement membrane zone antibodies can be co-expressed with IgG antibodies no positive IgA basement membrane zone antibody reactivity is detected by indirect immunofluorescence to indicate this or to support a diagnosis of linear IgA disease.

The negative IgG, including IgG4, and IgA cell surface (CS), also known as intercellular substance (ICS), antibody reactivity by indirect immunofluorescence is against, but does not rule out, the diagnoses of pemphigus vulgaris, pemphigus foliaceus, other IgG pemphigus variants, and IgA pemphigus. The normal IgG desmoglein 1 and IgG desmoglein 3 antibody levels by ELISAs also are against, but do not rule out, the diagnosis of active pemphigus foliaceus or pemphigus vulgaris.

Detection, levels, and patterns of diagnostic antibodies may fluctuate with disease manifestations, and IgG BP180 antibody levels correlate with disease activity in some patients with pemphigoid. Clinical correlation is needed, including with direct immunofluorescence findings on a biopsy specimen and treatment status. Monitoring antibody profiles by indirect immunofluorescence and antibody levels by ELISAs may be useful in assessing disease activity and expression, including response to therapy.

General

Approximately 80 percent of patients with bullous pemphigoid, epidermolysis bullosa acquisita, and linear IgA bullous dermatosis have positive antibodies to basement membrane zone components in their sera detected by indirect immunofluorescence. IgG4 subclass reactivity by indirect immunofluorescence may be more sensitive than IgG in some patients with pemphigoid and epidermolysis bullosa acquisita. Approximately 50 percent of patients with mucous membrane/cicatricial pemphigoid demonstrate antibodies to basement membrane zone components detected by indirect immunofluorescence. The immunoglobulin class of basement

membrane zone antibodies and pattern of antibody localization on split skin substrate distinguish the diseases. Positive serum IgA epithelial basement membrane zone antibodies are highly specific diagnostic markers for linear IgA disease. IgA basement membrane zone antibodies by indirect immunofluorescence may be found in variant presentations of mucous membrane pemphigoid and epidermolysis bullosa acquisita. Moreover, IgA basement membrane zone antibodies may be co-expressed with IgG basement membrane zone antibodies in some patients with pemphigoid including mucous membrane/cicatricial pemphigoid and, characteristically, in linear IgA/IgG bullous dermatosis.

Greater than 80 percent of patients with pemphigus have positive epithelial cell surface (CS) antibodies, also known as intercellular substance (ICS) antibodies, in their sera identified by indirect immunofluorescence. IgG4 subclass reactivity by indirect immunofluorescence may be more sensitive than IgG in some patients with immunobullous diseases. Serum antibody titers correlate with disease activity, and CS/ICS antibodies may be in low titer or negative in patients whose disease activity is minimal and/or under therapeutic control. Cell surface antibodies are implicated in the pathophysiology of pemphigus. IgG CS/ICS antibodies characteristically are positive by indirect immunofluorescence in IgG pemphigus variants, including pemphigus foliaceus and pemphigus vulgaris. IgA CS/ICS antibodies are positive by indirect immunofluorescence in patients with IgA pemphigus, although IgA CS/ICS antibodies may be observed in some pemphigus variants along with positive IgG CS/ICS antibodies. Approximately 40 percent of patients with nonclassical IgG/IgA pemphigus have an underlying systemic disease when diagnosed, malignancy being the most common.

Major molecular structures in the basement membrane zone to which IgG pemphigoid antibodies bind have been identified and termed "BP180" for a 180 kDa bullous pemphigoid antigen (also known as bullous pemphigoid antigen 2, BPAG2, or type XVII collagen, COL17) and "BP230" for a 230 kDa bullous pemphigoid antigen (also known as bullous pemphigoid antigen 1, BPAG1). BP180 is a transmembrane component of the basement membrane zone with collagen-like domains; the non-collagenous 16A (NC16A) antigenic domain of BP180 has been identified as a main antigenic target. BP230 is located in the hemidesmosomal plaque of basal cells in the epidermis. Serum levels of IgG BP180 and IgG BP230 antibodies are determined by ELISA, and serum levels of IgG BP180 antibodies may correlate with disease activity in pemphigoid, diminishing with treatment response. Up to 7 percent of individuals who do not have pemphigoid, including patients with other immunobullous diseases, have increased levels of IgG BP180 and/or BP230 antibodies by ELISAs. Patients with pemphigoid may show reactivity to multiple basement membrane zone components in addition to or other than the BP180 and BP230 epitopes expressed in the tested ELISAs.

Type VII collagen is a component of anchoring fibrils within epithelial basement membrane zone (skin and mucous membranes), and patients with epidermolysis bullosa acquisita characteristically develop IgG antibodies to type VII collagen. An increased serum IgG type VII collagen antibody level by ELISA provides support for the diagnosis of epidermolysis bullosa acquisita and also a subset of bullous lupus erythematosus together with dermal localization (floor) of IgG basement membrane zone antibodies on split skin substrate by indirect immunofluorescence. Patients with inflammatory bowel disease, including Crohn disease and ulcerative colitis, with and without mucocutaneous manifestations of epidermolysis bullosa acquisita, may demonstrate increased levels of antibodies to type VII collagen. The major epitopes for antibody reactivity reside in the non-collagenous amino-terminal domain, NC1, with minor epitopes in the non-collagenous carboxy-terminal domain, NC2, of the three identical alpha chains that comprise type VII

collagen. The tested ELISA contains combined purified recombinant antigens from both NC1 and NC2 for detection of IgG antibodies. Serum antibody levels above the reference range threshold of 6 U/mL may correlate with disease activity. Patients with epidermolysis bullosa acquisita or bullous lupus erythematosus may develop antibodies to basement membrane zone antigens in addition to or other than the type VII collagen epitopes displayed in this ELISA, and patients with other epithelial antibody-associated disorders may develop overlapping basement membrane zone antibody expression with an increased level of IgG type VII collagen antibodies.

Pathogenic antibodies in serum from patients with pemphigus bind to desmogleins, calcium-dependent adhesion molecules in cell surface desmosomes; such antibodies are detected by ELISA. Specific reactivity to the type of desmoglein may be helpful in determining pemphigus subtypes; the IgG desmoglein 1 antibody level is increased in patients with pemphigus foliaceus, and the IgG desmoglein 3 antibody level, with or without an increased IgG desmoglein 1 antibody level, is predominantly increased in patients with pemphigus vulgaris. Overlapping expression with antibodies to both desmogleins 1 and 3 clinically is associated with both mucosal and skin lesions. ELISA testing for IgG desmoglein 1 and IgG desmoglein 3 antibodies is highly sensitive, with greater than 90 percent of patients with pemphigus showing increased levels of one or both antibodies, and IgG desmoglein antibody levels correlate with disease activity. However, patients with CS/ICS antibody-positive pemphigus by indirect immunofluorescence can have normal results on ELISA testing with cell surface antibodies to different desmoglein 1 and/or desmoglein 3 epitopes than displayed in tested ELISAs or to other adhesion molecules.

TESTING METHODS

Indirect Immunofluorescence (IIF)

IgG, IgG4, and IgA Epithelial Basement Membrane Zone (BMZ) and Cell Surface (Intercellular Substance or ICS) Antibodies

Patient serum is progressively diluted beginning at 1:5 in four two-fold screening dilutions, layered on sections of human skin split at the basement membrane zone, intact human skin and monkey esophagus substrates, and reacted with fluorescein isothiocyanate (FITC)-conjugated antibodies to IgG and IgA. When positive, the serum is further diluted in two-fold reductions to the limiting dilution of antibody detection or to a maximum dilution of 1:40,960. The limiting-dilution, end-point titer is reported for each substrate, and the pattern of staining on split skin substrate also is reported. FITC-conjugated anti-IgG4 is tested to increase test sensitivity (maximum serum dilution of 1:40). This indirect immunofluorescence testing was developed and its performance characteristics determined by the Immunodermatology Laboratory at the University of Utah. It has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for this testing performed in a CLIA-certified laboratory (Clinical Laboratory Improvement Amendments) and intended for clinical use. [Indirect immunofluorescence, three antibodies on three substrates (IIF X 9) with two limiting dilution, end-point titers (antibody titer X 2)]

Enzyme-Linked Immunosorbent Assay (ELISA)

IgG BP180 and IgG BP230 serum antibody levels determined by U.S. Food and Drug Administration (FDA)-approved ELISAs (Mesacup, MBL BION). [Two ELISAs]

IgG type VII collagen serum antibody level determined by ELISA (Mesacup, MBL International). The performance characteristics of

this ELISA testing were determined by the Immunodermatology Laboratory at the University of Utah. The testing has not been cleared or approved by the FDA (US Food and Drug Administration). FDA clearance or approval currently is not required for this testing performed in a CLIA-certified laboratory (Clinical Laboratory Improvement Amendments) and intended for clinical use. [One ELISA]

IgG desmoglein 1 and IgG desmoglein 3 serum antibody levels determined by U.S. Food and Drug Administration (FDA)-approved ELISAs (Mesacup, MBL BION). [Two ELISAs]

Electronically signed by , MD, on
at .
Performed At:

Medical Director: , MD
CLIA Number:

VERIFIED/REPORTED DATES

Procedure	Accession	Collected	Received	Verified/Reported
EER Immunobullous Disease Panel	22-172-119076			
Immunobullous Disease Panel	22-172-119076			

END OF CHART

H=High, L=Low, *=Abnormal, C=Critical